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High field NMR analysis of the degree of substitution in carboxymethyl cellulose sodium salt

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Abstract

An accurate high field (^{1}H at 600.13 MHz) 1D and 2D study was performed on an aqueous solution of carboxymethyl cellulose (CMC) sodium salt at 90°C. A combination of 2D experiments was used, ^{1}H–^{1}H totally correlated spectroscopy TOCSY, and reverse detected ^{1}H–^{13}C hetero-correlated spectroscopy HMQC with gradient selection. This combination allows a full assignment of ^{13}C NMR spectra. From the gated decoupled ^{13}C spectrum it is then possible to obtain not only the degree of substitution but also a good measure of the amount of esterification in each chemical position. Conductimetric titration supports the obtained results.

Keywords: Carboxymethyl cellulose; NMR; Substitution

1. Introduction

Carboxymethyl cellulose (CMC) is a widely used polymer with many industrial applications but mostly used in food chemistry (Sanford & Baird, 1983). Its degree of substitution was traditionally determined by full degradation of the polymer followed by an analysis of the obtained monomers (Ho & Klosiewitz, 1980; Parfondry & Perlin, 1977; Reuben & Conner, 1983).

The method is imprecise and cumbersome. An improved method consisted in the direct analytical determination of the degree of substitution by ^{13}C NMR (Chaudhari, Gounden, Srinivasan & Ekkundi, 1987). The method is sound, but the reported spectra appear poorly resolved and the assignment seems only a tentative one.

Being interested in a full characterization of the title compound we performed a series of high field NMR experiments obtaining not only an accurate measurement of the degree of substitution, but also a good characterization of the degree of substitution in each chemical position. This task was performed through the following spectra, all obtained at 90°C in a D_2O solution 0.1 M in NaCl: 1D ^{1}H (600.13 MHz) and ^{13}C (150.91), 2D ^{1}H TOCSY (Kövér, Urhin & Hruby, 1998), and 2D ^{1}H–^{13}C HMQC (Hurd & John, 1991) hetero-correlated obtained by reverse detection, this sequence using gradient selection.

2. Experimental

Carboxymethyl cellulose sodium salt was purchased from Aldrich Chimica s.r.l. Milano, Italy, and used as received.

To bypass all resolution problems due to aggregation and the presence of both inter- and intra-molecular hydrogen bonds, we have chosen to operate at high ionic strength and at high temperature. Only one sample was necessary.

2.1. Conductimetric titration

For comparison purposes, determination of DS has also been performed by an alternative technique based on conductimetric titration.

According to this method, 0.5 g of dry CMCNa were dissolved in 500 ml of distilled water and stirred until a clear solution was obtained. To allow a reliable quantitative determination of the degree of substitution, all the carboxyl groups must be converted into their protonated form, before the titration is started. The solution of CMCNa was therefore acidified to pH = 3.2 by adding a solution of HCl 0.01 M.

At this point conductimetric titration with NaOH 0.025 M was performed at 25°C. During the analysis the solution was

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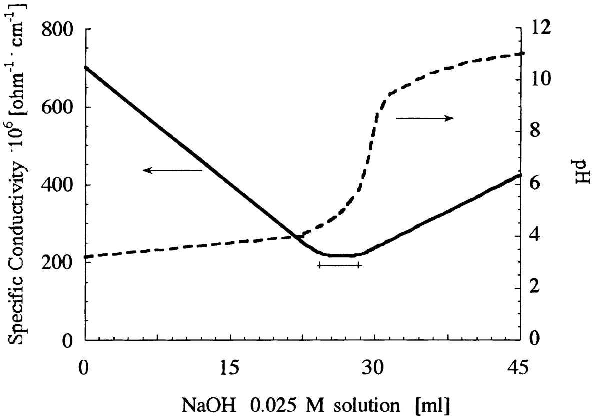

Fig. 1. Conductimetric titration.

maintained under stirring and purged with N_2; pH and conductivity were continuously measured by means of a pH-electrode and a conductimeter, respectively.

A typical plot reporting the change in conductivity and pH registered during the conductimetric titration is shown in Fig. 1. The first part of the curve, where the conductivity decreases rapidly, corresponds to the neutralization of H-ions coming from the strong acid added initially, the plateau observed just after is instead due to the neutralization of the weak carboxyl acid groups, while the final increase in conductivity corresponds to an excess amount of NaOH. From the measure of the plateau length, the amount of carboxylic groups per gram of CMCNa, and therefore the DS of the polymer, has been finally calculated.

2.2. Spectroscopy

All NMR spectra have been run at 90°C in D_2O (99.8% isotopic purity), 0.1 M in NaCl. ^1H NMR were performed at 600.13 MHz, ^{13}C NMR spectra were performed at 150.13 MHz. The following experiments were performed.

^1H (time domain 32 K), $^1H-^1H$ TOCSY, see Fig. 2; size 1024×512, water suppressed by signal pre-saturation, contact time 80 μs.

$^{13}C\{^1H\}$ performed both with a straightforward GARP (Bax, 1983) decoupling sequence as well with a decoupling sequence inverse gated (Freeman, Hill & Kaptein, 1972) zeroing all heteronuclear Overhauser effects, see Fig. 3.

$^1H-^{13}C$ correlated map (HMQC), performed with proton detection and gradients selection, see Fig. 4; size 1024×512, contact time chosen to optimize a $J_{H-X} = 140$ Hz, gradients along z-axis.

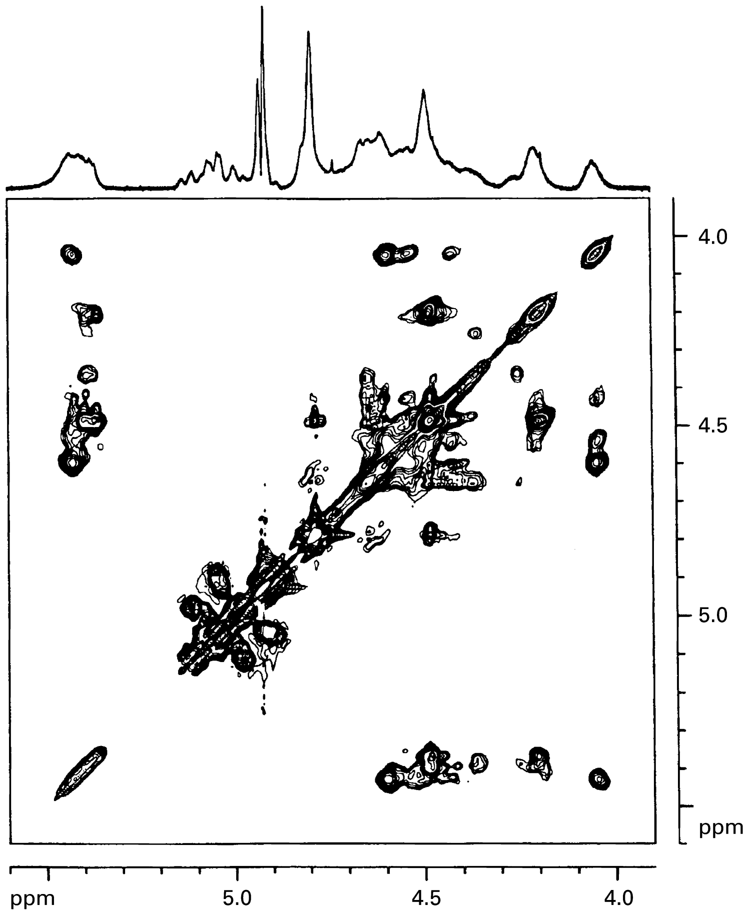

Fig. 2. $^1H-^1H$ 2D TOCSY map of CMC Na in D_2O + NaCl 0.1 M at 90°C; 1024×512. Mixing time 80 ms. Zero filled in F1 and Fourier transformed after a qsin (squared sinus) multiplication in both dimensions. In the top the ^1H spectrum is reported.

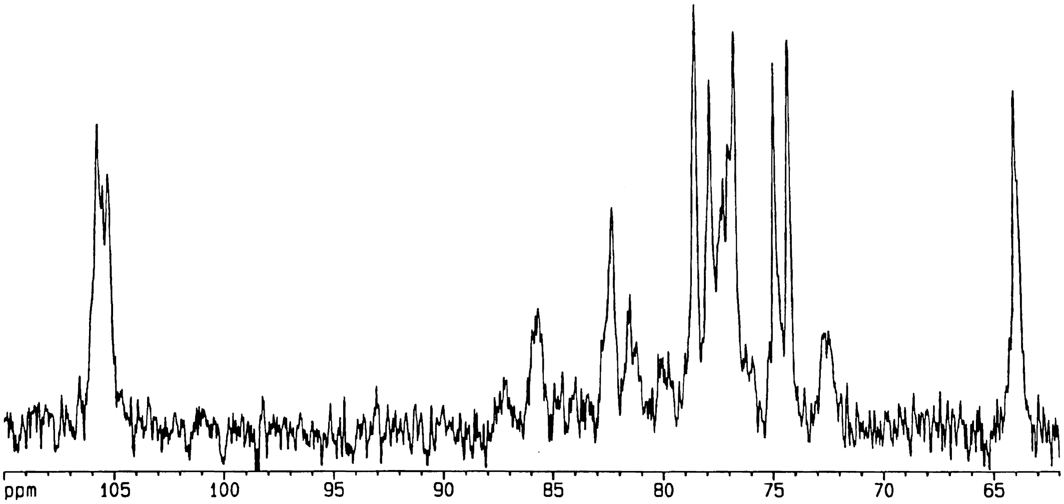

Fig. 3. $^{13}C\{^{1}H\}$ inverse gated decoupled spectrum, only the 60–110 ppm range is shown.

3. Results and discussion

Let us take into consideration a whatsoever chemical reaction leading to a functionalization of an alcoholic group on a cellulose matrix. Let us also suppose the case of a functionalization reaction occurring in defect of the functionalizing reagent. In this case it is reasonable to hypothesize that only one alcoholic group reacts per monomer unit.

The reaction may occur in position 2,3,6 leading to a β-effect on the chemical shift of the involved C atom. Thus after the esterification we should expect a strong downfield chemical shift (Lindemann & Adams, 1971). This downfield shift should be additive, independent of the position and of the order of 8–10 ppm.

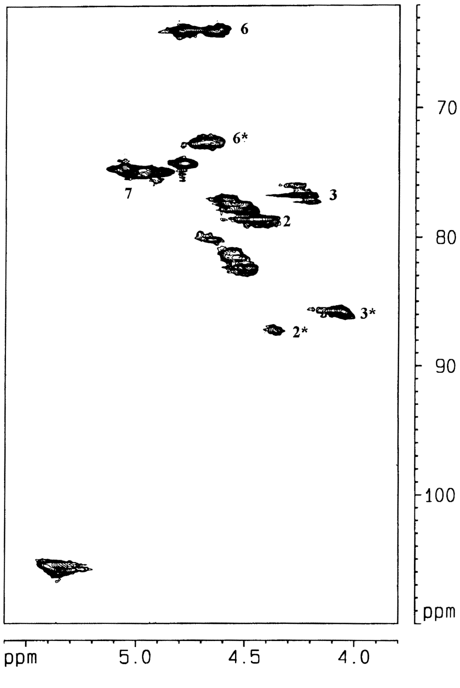

Fig. 4. ^{1}H–^{13}C HMQC map. Mixing time corresponding to a *J* coupling of 140 Hz; 1024 × 512. Zero filled in F1 and Fourier transformed after an exponential multiplication in both dimensions (line broadening 4 and 8 Hz, respectively, in F2 and F1 dimensions).

Let us mark with a * the position in which the reaction has occurred which can be 2 or 3 or 6, see Table 1.

In the NMR ^{13}C spectra of the functionalized cellulose another well observable effect should be observed, this is due to γ-, δ-, ϵ-effects occurring in the same ring in which the substitution has occurred. These intra ring effects are well documented and can lead to both downfield or upfield effects, of minor entity with respect to the direct β-effect but still well observable and of the order of ±1–2 ppm.

Inter-ring effects, even when present, can be supposed much smaller and in the case of a high molecular weight polymer able to produce only a broadening of the ^{13}C resonances.

Finally let us consider the O–CO–CH_2–O resonance. Both in the ^{1}H and in the ^{13}C spectra we should expect different chemical shifts for the three possible positions. We should also expect a chemical non-equivalence of methylene protons, possibly larger in the case of a substitution on position 2 or 3 in respect to a substitution in 6.

Both methylene and carboxyl C atoms should strongly resent upon the position of the substitution. We will not discuss the intensity of the carboxyl resonance since this value is strongly affected by spurious effects such as concentration and ionic strength. Moreover, the presence of long relaxation times and the absence of direct Overhauser effects, even operating in the inverse gated mode (Freeman et al., 1972), weakens the intensity of the carboxyl resonance, so that we thought it better to perform our study using all other resonances of the ^{1}H and ^{13}C spectra.

Let us briefly discuss the obtained assignment. The spin system belonging to the O–CO–CH_2–O group is never TOCSY coupled to any proton belonging to the ring spin system. Three AB spin system can be easily identified:

Table 1
Carboxymethyl cellulose Na salt (CMC Na), ^{1}H and ^{13}C chemical shifts (substitution on C*; methylenic carbon atom CO–CH$_2$–O– labeled as 7; carbonyl group, not assigned to individual residues, in the range 180.0–181.5)

C1, H1	C2, H2	C3, H3	C4, H4	C5, H5	C6, H6,6$'$	C7, H7,7$'$
105.3, 5.37	78.65, 4.53	76.78, 4.21	82.32, 4.48	77.95, 4.49	63.96, 4.64–4.80	
				80.0, 4.63	C6* 72.75, 4.72–4.65	74.27, ≈ 4.78
105.8, 5.42	≈ 77.0, 4.60	C3* 85.85, 4.05	81.51, 4.57	≈ 77.5, 4.43		75.06, 5.05–4.91
105.6, 5.40	C2* 87.29, 4.36	≈ 83.9, 4.26				74.82, 5.10–5.05

5.10–5.05 ppm; 5.05–4.91 ppm and 4.78 (with a small AB chemical shift of the order of 2 Hz); they correspond to a methylenic carbon atom, respectively, at 74.27, 75.06 and 74.82 ppm, see Table 1. Not-substituted cellulose can be easily reckoned and its ^{13}C resonances assigned.

The HMQC experiment evidences three resonances, respectively, at about 9 ppm from C6, C3 and C2 of the corresponding resonance of not-substituted cellulose. These resonances were tentatively assigned to C6*, C3* and C2*. The TOCSY experiment confirmed this assignment and gave the chemical shift values of resonances due to protons not bearing any substitution but belonging to a ring, respectively, substituted in 6, 3 or 2. Thus, combining the information of all these experiments we obtained the assignment given in Table 1.

The above assignment is not inconsistent with a previously proposed ^{13}C assignment (5); it is however more comprehensive. The main discrepancy regards some resonance now attributed to CH$_2$ in position 7, which is assigned on the basis of TOCSY experiments followed by the hetero-correlated 2D map.

4. Conclusions

From the integrals performed on the ^{13}C spectrum obtained in the gated decoupling mode, it appears evident that it is possible to obtain not only the degree of substitution, which appears to be ≈ 0.65–0.68 per monomer unit, but a good measure of the amount of esterification in the different positions. This turns to be ≈ 40% in position 3, ≈ 40% in position 6 and ≈ 20% in position 2.

A corresponding measure can be done on the ^{1}H spectrum, where the errors on the integrated intensities are higher due to the presence of the HOD resonance, very near to the group of resonances attributed to the CH$_2$ in position 7. However, with the ^{1}H attribution shown in the table the two sets of data appear quite consistent.

The DS measured by means of conductimetric titration, a more traditional but evidently more cumbersome technique, is 0.66 ± 0.03 showing a very good agreement with the results obtained by using the new and experimentally easier high field NMR technique illustrated in this paper.

Our paper is meant to give a good NMR characterization of the title compound. This is necessary before any further study such as crosslink determination, hydrogen bonding system and aggregation.

Acknowledgements

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